Minor neurological signs and behavioural function at age 2 years in neonatal hypoxic ischaemic encephalopathy (HIE)

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Abstract

Background. Neurodevelopmental follow-up in Neonatal Hypoxic Ischaemic Encephalopathy (HIE) typically focusses on major neuromotor (cerebral palsy, CP) and severe cognitive impairment. Outcomes in those without major neuromotor impairment are less well explored.

Objectives. To examine behavioural, cognitive and neurological outcomes after neonatal HIE, in a clinical cohort of children without CP, at age 2 years.

Methods. Clinical routine outcome data from children admitted to a tertiary centre with neonatal HIE for hypothermia treatment between 05/08/09 - 30/05/2016. Children were assessed for neuromotor status – particularly minor neurological signs (MNS), with Bayley Scales of Infant and Toddler Development III (Bayley III) or Ages and Stages Questionnaire-3 (ASQ), Child Behavior Checklist 1.5-5 (CBCL), Quantitative Checklist for Autism in Toddlers (Q-CHAT).

Results Of 107 children, 75.5% had normal neurology, 12.1% CP, 12.1% MNS. Children with CP were excluded from analyses. For those without CP, Bayley-III scores were in the average range for the majority; mild cognitive delay observed in 5%, 4.2% language, 1.3% motor development; severe delay in 1.3% for cognitive, 4.2% for language. More than in the normative population scored in clinical ranges for CBCL externalising, sleep, and other problems. No significant difference was seen for Q-CHAT. Children with MNS were significantly more likely to have impaired Bayley-III scores, parent-reported internalising, sleep, and other problems.

Conclusions. In this clinical cohort, the majority of children had favourable outcome at 2 years. However, children with MNS were at risk for cognitive and behavioural difficulties and will benefit from enhanced clinical follow-up and support.

Key words

Neonatal Hypoxic Ischaemic Encephalopathy (HIE)

Therapeutic Hypothermia

Minor Neurological Signs

Neurodevelopmental

Neuromotor

Abbreviations:

Hypoxic Ischaemic Encephalopathy (HIE)

Cerebral Palsy (CP)

Therapeutic Hypothermia (TH)

The Bayley Scales of Infant and Toddler Development, 3rd edition (Bayley-III)

Ages and Stages Questionnaire 3 (ASQ)

Child Behavior Checklist 1.5 - 5 years (CBCL)

Quantitative Checklist for Autism in Toddlers (Q-CHAT)

Minor neurological signs (MNS)

Minor Neurological Dysfunction (MND)

Movement Assessment Battery for Children-2 (M-ABC)

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1. Introduction

Neonatal Hypoxic Ischaemic Encephalopathy (HIE) as a consequence of peripartum asphyxia is a major cause of neurological injury, affecting 1.3-1.7 newborns per 1000 live births in middle to high income countries [1]. Children surviving neonatal HIE are at increased risk of adverse outcomes including severe neuromotor impairment (cerebral palsy, CP), global cognitive impairment, visual and/or hearing impairment, and epilepsy.

Therapeutic hypothermia (TH) has now become standard care for infants with moderate or severe HIE. Several large randomized controlled trials [2–4] have shown that TH reduces both mortality and severe neurodisability. These effects appear to continue to school age [3–5], although there are some inconsistent results between studies with regards to neurodevelopmental outcomes.

The British Association of Perinatal Medicine and The National Institute for Health and Care Excellence (NICE) currently recommend that a formal neurodevelopmental assessment is carried out at around 2 years of age in children surviving neonatal HIE. These assessments typically comprise a developmental assessment such as the Bayley Scales of Infant and Toddler Development, but information about behavioural impairments or social and emotional functioning is rarely reported. After this, the children are typically discharged from clinical follow-up unless they have global developmental or severe neuromotor impairment (CP).

Studies examining long term outcomes have typically focused on outcomes of HIE such as CP and global cognitive impairment There is little information [6,7] on whether there are specific groups of children surviving HIE without major neuromotor impairment that may be at heightened risk of cognitive and/or behavioural impairment, and the majority of information dates from the period prior to TH becoming routine clinical practice. However, some studies have shown that even in the absence of such global impairments [8–10] children

with HIE may also exhibit motor, cognitive and behavioural impairments [11,12], and a recent systematic review [13] indicates that in the absence of CP, a high proportion of survivors of HIE remain at risk of general and/or specific cognitive impairments, even after TH. Current evidence for behavioural problems is limited [13] .

Thus, this indicates that it is important to assess cognitive and behavioural outcomes of children with HIE with and without CP separately. In the present study, which investigates a clinical single centre cohort, we therefore focus on those children who have survived without developing severe neuromotor impairment. The aim of the current study is to describe the neurological, cognitive, and behavioural outcomes at a developmental age 2 years of a clinical sample of children who were admitted to a Neonatal Intensive Care Unit (NICU) for consideration of hypothermia treatment for HIE.

2. Methods

Secondary analysis of anonymised routinely collected clinical data data on this sample of children was approved by the University of Southampton Research Ethics Committee (Ethics ID: 26356) and the HRA and Health and Care Research Wales, HCRW (Reference ID 20/HRA/0260; IRAS project ID 278072; University Hospital Southampton R&D protocol number RHM CHI1047).

2.1. Study Population

Infants were enrolled in the study if they had been admitted to the neonatal unit at University Hospital Southampton between 05/08/09 to 30/05/2016 for consideration of hypothermia treatment and enrolled in the clinical follow-up programme. Exclusion criteria were causes for encephalopathy other than perinatal asphyxia, genetic or syndromal disorders and not

receiving the full 72 hours of TH. Our criteria for TH are: gestational age ≥36 weeks (however, in this clinical cohort, there were 3 infants who were born <36 weeks, 1 at 34 weeks, and 2 at 35 weeks of gestation, who received TH) and at least one of the following: Apgar score of 5 or less 10 min after birth; continued need for resuscitation, including endotracheal or mask ventilation, 10 min after birth; or acidosis (defined as pH <7 or base deficit >15 mmol/L, or both, in umbilical cord blood or any blood sample) within 1 h of birth [14], and showed signs of moderate to severe encephalopathy. Severity of encephalopathy was classified using the modified Sarnat and Sarnat [15] staging; altered state of consciousness (reduced or absent response to stimulation), abnormal tone, and abnormal primitive reflexes (weak or absent suck or Moro response). In line with clinical protocol of this centre which is set to avoid delay in initiation of TH, amplitude integrated EEG (aEEG) was not used to determine initiation of TH.

2.2. Neurodevelopmental Assessment

Children were assessed in the follow-up clinic using a standardised protocol carried out by a Paediatric Neurologist or Neonatologist with experience in neurological and developmental assessments, together with a Physiotherapist.

2.2.1. Structured neurological examination

Neurological examination included assessment of cranial nerve function, movements, posture, reflexes, and muscle tone. Neurological status was categorised as normal (completely normal neurologic status), minor neurological signs (gross or fine motor coordination difficulties, muscle tone imbalance, without definite signs of cerebral palsy [CP]), or abnormal (signs of CP present as defined by the Surveillance of Cerebral Palsy in Europe Working Group, SCPE, 2000 [16]). Assessors were not blind to the neonatal course since the children were assessed in a follow-up clinic for infants who had neonatal HIE.

2.2.2. Developmental Assessment

2.2.2.1. Bayley Scales of Infant and Toddler Development –III

The Bayley Scales of Infant and Toddler Development –III (Bayley-III) [17] is a standardised assessment which consists of a series of developmental play tasks. Composite scores are derived for cognitive, language, and motor development and scaled to a metric, with a mean of 100, standard deviation of 15, and range of 40 to 160. Mild impairment was defined as a composite score ≥1 – 1.5 SD below the mean, and severe impairment as a composite score ≥2 SD below the mean.

2.2.2.2. Ages and Stages Questionnaire-3 (ASQ)

Where the Bayley-III could not be completed due to non-compliance, parents completed the Ages and Stages Questionnaire-3 (ASQ) instead. The ASQ is a questionnaire that screens children for development in the areas of communication, gross and fine motor development, personal-social development, and problem-solving skills. It can be used for children aged between one month and 5½ years. The ASQ has been found to have similar predictive value for follow-up decisions to the Bayley-III [18].

2.2.3. Behaviour including attention and autism spectrum disorder symptoms

2.2.3.1. Child Behavior Checklist 1.5 -5 (CBCL)

The parental version of the Child Behavior Checklist 1.5-5 (CBCL) was used to screen for behavioural difficulties. The CBCL 1.5-5 uses parental and/or teacher behavioural ratings to detect emotional and behavioural problems in preschool-aged children. It provides normed scales of Internalising, Externalising, and Total Problems derived from subscales including emotionally reactive, anxious/depressed, withdrawn behaviour, somatic complaints, for the

internalising scale; attention problems and aggressive behaviour for the externalising scale; sleep and other problems [19]. As recommended by Achenbach & Edlebrock (1993) [20], we used raw scores in the analysis as they are more precise and uniform than t-scores. The normative sample of the CBCL is based on data from parental report of 700 healthy children from 40 different states in the United States.

2.2.3.2. Quantitative Checklist for Autism in Toddlers (Q-CHAT)

The Q-CHAT is a 25-item parental report of autistic behaviour which assesses domains of joint attention, pretend play, language development, repetitive behaviours, and other aspects of social communication. The psychometric properties and predictive value of Q-CHAT have been previously examined [21] and the tool has a range of scores which approximate normal distribution [22]. The Q-CHAT is able to discriminate between toddlers with and without a diagnosis of autism, however, to date, a cut-off point above which children would be invited for further assessment is yet to be validated. To evaluate the Q-CHAT scores in the present study, the mean +2SD for the normative population reported in Allison and colleagues' paper (mean for normative population 26.7, SD 7.8; boys 27.5, SD 7.8; girls 25.8, SD 7.8) were used as a comparison [22].

2.3. Statistics

Outcomes are first reported across the whole sample of tested children, then comparisons are made between children with minor neurological signs (MNS) and normal neurology. Children with a diagnosis of Cerebral Palsy are excluded from these comparison analyses. Statistical tests were conducted using IBM SPSS Statistics 25. Differences between groups were assessed with independent samples t-test where data were normally distributed and continuous and Mann-Whitney U test where the data were non-normally distributed and

continuous, and with Fisher's Exact tests for categorical data (as some cells contained fewer than 5 samples). Comparisons between group means and published norms were made using a one sample t-test. Where categories were determined from continuous data e.g. mild or severe delay on assessment with the Bayley Scales, or borderline or clinical range in CBCL, reference values given in the test manuals were used.

3. Results

As shown in Figure 1, 194 newborns were admitted for TH between 05/08/09 to 30/05/2016. Seventeen were excluded from the present analysis as they had a primary diagnosis other than HIE due to perinatal asphyxia; 17 (8.8%) infants were excluded since they did not receive TH or TH was not administered for the full 72 hours since the treating clinician either felt after review that TH was not indicated at all, or could be stopped before 72 hours of treatment. Of the 140 infants who had a primary diagnosis of perinatal asphyxia as a cause for encephalopathy and who received 72 hours of TH, 13 (9.3%) died. Of the 127 surviving newborns, 20 (15.7%) were lost to follow up, 107 (84.3 % of the survivors) received follow-up at age 2 years. Of those, 87 (81.3%) were assessed according to our standardised neurodevelopmental follow-up protocol (see below), and information on neurological outcome was available for further 20 (18.7%) children who were seen by their Neonatologist or Paediatrician for follow-up but were not formally assessed by the Paediatric Neurologist or according to the standardised protocol. The mean age of the tested children was 25.9 months (SD 2.6 months; min 21 - max 32.1 months). The baseline characteristics of the surviving infants for whom follow-up data were available did not differ significantly from those who were lost to follow-up (Table 1).

3.1. Neuromotor outcomes

Of the 107 children with information on neuromotor outcome, 81 (75.7%) had normal neurology, 13 (12.1 %) had minor neurological signs, and 13 (12.1 %) had CP. The children with a diagnosis of CP were excluded from subsequent analyses, leaving a final sample for analysis of 94 children.

3.2. Developmental outcomes

Analyses of developmental outcomes were performed on the sample of children who had not developed CP by the age of 2 years (n=94). 71 of the 94 children (75.5 %) were assessed with the Bayley-3 Scales and in 10 (10.6%) developmental screening was performed with the ASQ-3. For 13 (13.8%) of the children without CP only information on neurological status was available.

3.2.1. Bayley-III Scales

For children (without CP) tested with the Bayley-III Scales (n=71), mean Bayley composite scores were broadly average, see Table 2. When considering the presence of delay, 94.4% of tested children had normal cognitive scores (>85), 4.2% mild cognitive delay (70-85), and 1.4% severe cognitive delay (<70); 92.2% had normal language scores (>85), 3.1% mild language delay (70-85) and 4.7% severe language delay (<70); and 98.5% had normal motor scores (>85), 1.5% mild motor delay (70-85), none had severe motor delay (<70).

3.2.2. Ages and Stages Questionnaire-3

Ten children had developmental screening with the ASQ. The majority of children in this subgroup had scores in the normal range for all areas that were assessed (for communication 10/10; gross motor development 8/10; fine motor development 9/10, problem solving skills development 9/10, and personal-social development 9/10). Eight of the 10 children had

normal neurology, and 2/10 had MNS. Visual inspection of the date indicated that there was no obvious difference between those with normal neurology and those with MNS (see Table 3). However, it has to be kept in mind that the sample size was very small.

3.2.3. Assessment of behaviour

3.2.3.1. Screening for autism spectrum symptoms with the QCHAT

Information from the QChat was available for 71 (75.5%) of the 94 children without CP. The mean score across all tested children was 28.0 (SD 9.4; min 12-max 56). This was not significantly different from the published norm of 26.7 (Allison et al., 2008); one sample t-test, t (0.97), mean difference = 1.05, p = 0.33). There was weak evidence (p=0.07) for those with MNS (n=10; mean score 32.7, SD 10.4) showing more autistic spectrum disorder symptoms than those with normal neurology (n=61; mean score 26.9, SD 8.6).

3.2.3.2. Screening for internalising, externalising, sleep and other problems with the CBCL

Information from the CBCL was available for 74 (78.7 %) of the 94 children without CP. Table 4 shows the mean scores for total problem scale, internalising and externalising problems, sleep problems, and other problems. On the CBCL, 72.9% of children had total problem scores in the normal range, 10.8% in the borderline range, and 17.6 % in the clinical range. For internalising problems, 85.1 % scored in the normal range, 5.4% were in the borderline clinical range and 9.5% in the clinical range. For externalising problems, 74.3% of children scored in the normal range, 6.8% in the borderline clinical range, and 18.9% in the clinical range. Clinically relevant sleep problems were reported for 8.4% of the children, borderline scores for 7.3%, and 84.3% had scores in the normal range for sleep behaviour. In comparison, normative published means show that, for total problems, externalising and internalising scores, 82% of children scored in the normal range, 8% in the borderline range,

and 10% in the clinical range. For sleep problems, normative means report that 92% of children score in the normal range, 5% in the bordeline range and 3% in the clinical range [20].

Comparing the group with MNS (n = 10) with those with normal neurology (n = 64), total problem scores were significantly (p = 0.04; Table 4) higher in the group with MNS. Looking at the component parts of the overall score, there was a significant difference between groups for reported sleep problems (p = 0.04), "other problems" (p = 0.01) and internalising problems (p = 0.03). There was no difference between those with MNS and normal neurology for externalising problems (p = 0.09). The observed differences between the two groups on the internalising scale were most pronounced for the subscales screening for anxious/depressed behaviour (p=0.04), somatic complaints (p = 0.07), emotionally reactive behaviour (p = 0.06). For the externalising scale, there was weak evidence that aggressive behaviour was more frequent that in those with normal neurology (p = 0.06).

4. Discussion

When the whole sample of 2 year old children with HIE who survived without developing CP are considered, the majority of children scored in the normal range on cognitive, motor and language assessments. CBCL total scores in children with HIE prior to TH becoming standard clinical care report similar overall rates of total problems to those observed in our study, but the breakdown of total problems score into the subcomponents was not reported [23]. Our data add to the literature on the particular types of behavioural problems that children with HIE treated with TH might experience. In our sample, the proportion of children experiencing internalising problems was similar to that observed in the general population (when compared to test norms); our data suggest that high rates of overall problems on the CBCL total problems score are specific to difficulties with externalising problems and sleep.

The pattern of findings for performance on the Bayley Scales were similar, with composite scores similar to those reported in the general population. It is tempting to speculate that TH may not be protective with regards to more subtle behavioural problems. However, current evidence is limited, and more research is required here.

In our cohort, 75.7% of children were neurologically normal, 12.1% had CP (and were excluded from our analyses) and 12.1% had minor neurological signs. The rate of CP is lower than that reported for toddler age from the large RCTs on therapeutic hypothermia [24] but similar rates have been reported from observational studies [25]. Comparison of the rate of MNS is difficult, since this has not been previously reported in children with HIE treated with TH at toddler age. This, however, appears to be an important group since they were found to consistently experience more difficulties across multiple domains than the children with HIE and normal neurology. Children with MNS differed from children with normal neurology in multiple domains, with significantly lower cognitive, language and motor scores, as well as slightlyhigher ratings of autistic traits (Q-CHAT). Children with MNS scored higher on the total problem and internalising scales and had higher scores for parent reported sleep problems on the CBCL than children with normal neurology. Analysis of the individual subscales of the CBCL showed that children with MNS had higher scores in the domain of anxiety/depression, and this difference was approaching significance for emotionally reactive and somatic complaints subscales .

In the context of those children with MNS having lower cognitive scores, and screening for behavioural and sleep problems indicating more difficulties than for those with normal neurological examination, the concept of Minor Neurological Dysfunction (MND) is of interest. Minor neurological dysfunction, usually not diagnosed before school age, describes a

neurological profile that includes difficulties with muscle tone regulation, posture, balance, coordination, mildly abnormal reflexes and cranial nerve function [26]. Whilst the complex form of MND has been linked to learning, cognitive and motor problems in school aged children born very or extremely preterm [27,28], both the presence of MNS or MND, and whether it is linked to other aspects of cognitive and behavioural development, has not been previously reported in children with HIE. It has to be noted that the presence of MNS at toddler age does not necessarily mean that these children will go on to have MND later in life. The children in our cohort are too young for a formal assessment of MND with the currently available tools (i.e. Touwen Neurological Examination) and therefore it is not possible to assign a diagnosis of MND to the children in our cohort in whom MNS were seen. Currently, there is work going on for validation of an assessment for MND that can be used at toddler age (Hempel Assessment) to identify MND, but validity and reliability data are not yet available for the populations of infants with HIE or born preterm. However, it seems reasonable to infer from our findings of associations between MNS and poorer performance on cognitive assessment and behavioural difficulties' screening that those with MNS need enhanced surveillance and specific assessment for MND once they reach school age.

Comparison with other studies is difficult since, to our knowledge, no data on MNS at toddler age in the context of HIE have been reported. Some information is available for school age. Marlow et al (2005) [29], in a regional cohort of school aged children with neonatal encephalopathy (not treated with TH), using the Touwen assessment for MND, did not find an increased prevalence/incidence of MND in the absence of CP. However, this is the only study looking at MND in children with neonatal HIE and more research is needed to either replicate or disconfirm this, in particular for children who were born in the era of TH. Furthermore, at older ages, outcome assessments vary for the few existing studies, with most

studies focussing on assessment of motor function and not including assessment of minor neurological signs similar to that used in our study. However, comparison is possible with regards to occurrence of atypical neuromotor signs generally. Perez et al, 2013 [6], described for a cohort of school and teenage age children with a history of neonatal HIE (not treated with TH) who had survived without developing CP, impairment in motor speed and quality of movements, and this was related to impairment of general cognitive abilities. This is in line with our findings. No data on behavioural outcomes were, however, reported for this cohort. More recently, two papers were published on one small sample size cohort of 6-8 year of children who underwent TH. These studies reported that the total score on the Movement Assessment Battery for Children-2 (M-ABC) was significantly lower (indicating poorer motor performance) in children with HIE compared to controls [11,12], and associations between MABC-2 with Full Scale IQ, working memory and perceptual reasoning scores, with those showing poorer performance on motor tests performing poorer on the cognitive tests. One of the papers noted poor predictive value of Bayley-3 motor composite score at 18 months on MABC-2 scores at age 6-8 years, again indicating that long term follow up is essential [11].

Children with developmental difficulties can experience a widening gap on starting school, when cognitive and behavioural demands increase, and specific learning difficulties are more readily observed, as documented in the literature on school readiness [30,31]. Thus, it is important to assess the longer term developmental trajectory of children with HIE. A recent systematic review of the literature that focused on follow-up studies in cohorts aged 4 years or older reported that a large proportion of children with HIE without CP are at increased risk of cognitive impairment, with specific cognitive difficulties in attention, language and executive functions, and limited evidence for behavioural problems [13]. A wider range of more

focussed developmental assessments exist to assess school aged children and the presence of specific cognitive, emotional and behavioural difficulties should be followed up in future studies.

Comparison with another group of infants with early brain injury or atypical brain development, i.e. children born very or extremely preterm, shows a similar pattern of associations between neuromotor signs in the absence of CP and cognitive and/or behavioural function as in our cohort [27,28]. This might suggest that, despite injury to the brain occurring at different developmental stages in these two groups, subsequent subtle widespread alterations to similar brain networks involved in motor control may be present. To further examine this, MRI studies on anatomical and functional brain networks would be useful.

While the majority of children in our sample with HIE without CP scored in the normal range for sleep behaviour, the incidence of clinically relevant sleep problems was over twice that reported in typically developing children. Furthermore, in our study, significantly more sleep problems were reported in children with MNS compared to those with normal neurology. Other studies have also indicated problems with sleep behaviour in children with HIE. For example, Ding et al (2016) [32] reported that overall sleep times of 3-year-old children with HIE were shorter than those of typically developing, age-matched controls, potentially due to difficulties in sleep initiation and maintenance, as well as breathing problems during sleep. Additionally, there was an indication that different types of difficulties may be experienced by children with mild and moderate HIE. Sleep-related problems in children with HIE can be observed shortly after birth. Infants with HIE show a delay in development of the sleep-wake cycle measured using EEG [33,34], a delay which is related to the severity of HIE [33]. Furthermore, long-term developmental outcome was related to the onset of the sleep-wake

cycle; 1 to 5.5 year-old-children with HIE whose newborn sleep-wake cycle started before 36 hours had Griffiths scores 8.5 points higher than those whose newborn sleep-wake cycle started after 36 hours [33]. These findings should be followed up in a systematic manner to evaluate both the presence and type of sleep problems, and the relation to long-term outcome.

As TH is now standard treatment for moderate to severe HIE, contemporaneous comparisons of TH treated and non-treated children is no longer possible. Moving forwards, it may be of particular interest and relevance to compare children with HIE treated with TH with their age matched peers without HIE, in order to explore how they are performing later in life. With regards to assessment of neurology and neuromotor function, it will be important in future research to use specifically designed standardised and validated tools for detection of minor neurological dysfunction, combined with standardised and validated tests of neuromotor function.

Our findings have implications for clinical practice. Children surviving HIE who do not present with CP are often routinely discharged from clinical care at age 2 years. Children surviving HIE who present with MNS are rarely followed up clinically beyond 2 years of age. Furthermore, clinicians who perform follow-up assessments are not always trained to detect MNS. Our findings suggest that children surviving HIE with MNS are at heightened risk of impairment in multiple domains compared to children with HIE who exhibit normal neurology. This suggests that these children may benefit from a longer period of routine clinical follow up to monitor their development and to provide appropriate support.

Important limitations of our work include the relatively small sample size of the cohort and that not all children completed the follow-up assessment. As there were no significant

baseline differences between tested and not tested children, while possible, it seems unlikely that attrition bias could explain our findings.

5. Conclusion

In conclusion, in our clinical cohort of children with HIE without CP treated with TH, our two-year follow-up found that the majority of the sample showed typical development for cognition, motor skills, and language. There was no strong evidence of increased autistic traits, but more children exhibited externalising symptoms in the clinical range than in the general population. The subgroup of children with HIE and MNS consistently experienced greater difficulties across multiple domains than those with normal neurology, with difficulties in internalising behaviour, sleep, and other problems. Therefore, this group may benefit from enhanced clinical follow up to monitor their development, including sleep patterns, and to allow physicians to identify those who would benefit from early intervention. In addition, clinicians should be trained to identify minor neurological signs in these children and an appropriate care pathway should be designed.

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Figure 1: Study population. Children eligible for this study were admitted to Neonatal Unit for consideration of hypothermia treatment (TH) for neonatal HIE, University Hospital Southampton from 05/08/09 to 30/05/2016.

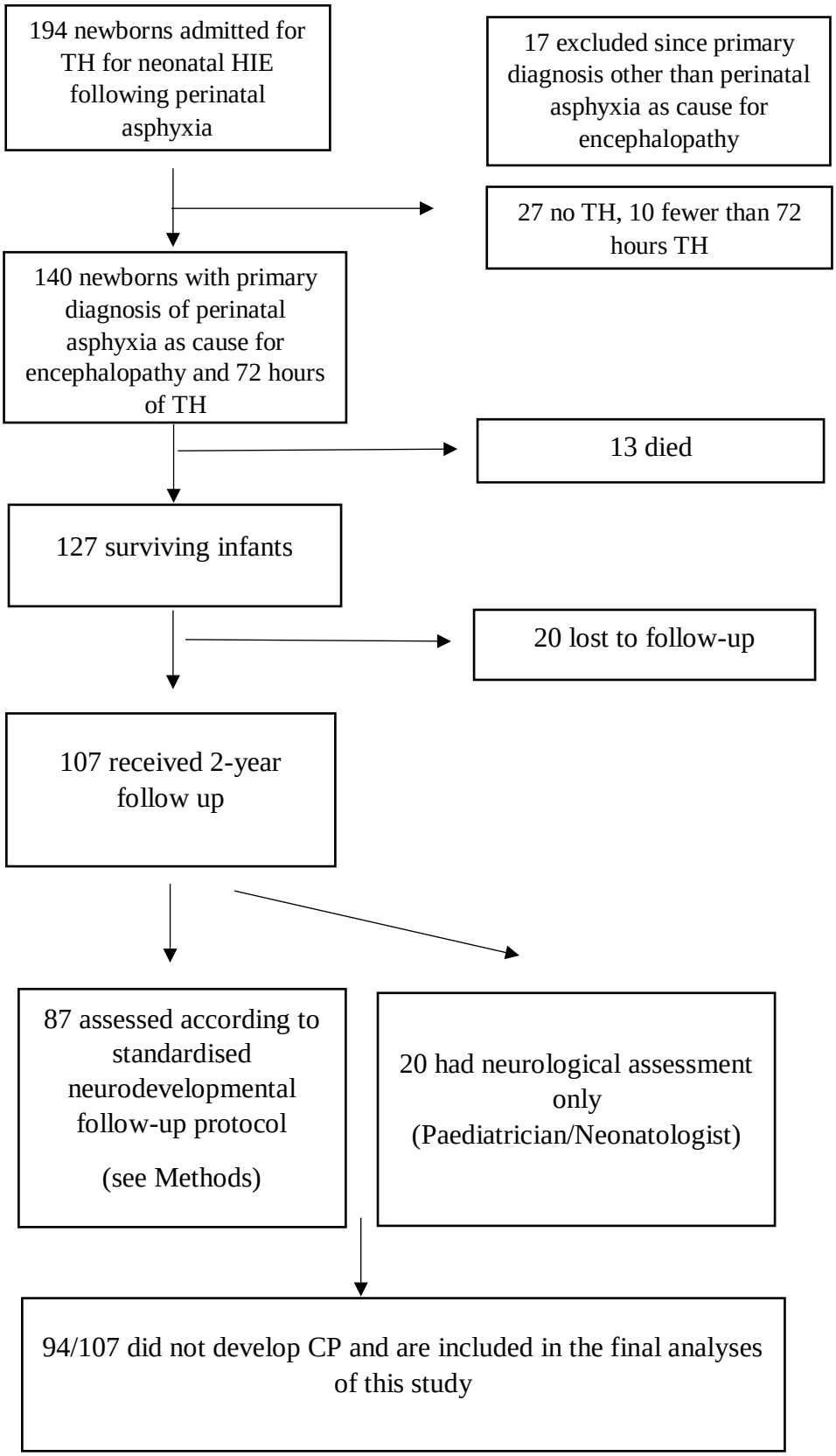
194 newborns admitted for TH for neonatal HIE following perinatal asphyxia
17 excluded since primary diagnosis other than perinatal asphyxia as cause for encephalopathy
27 no TH, 10 fewer than 72 hours TH
140 newborns with primary diagnosis of perinatal asphyxia as cause for encephalopathy and 72 hours of TH
13 died
127 surviving infants
20 lost to follow-up
107 received 2-year follow up
87 assessed according to standardised neurodevelopmental follow-up protocol
(see Methods)
20 had neurological assessment only
(Paediatrician/Neonatologist)
94/107 did not develop CP and are included in the final analyses of this study

Table 1: Demographic data for tested and untested surviving newborns with perinatal asphyxia and those treated with TH; note that this table excludes newborns who had other diagnoses than perinatal asphyxia as primary cause for encephalopathy and includes the children who developed Cerebral Palsy (CP)

	Whole Sample of newborns with perinatal asphyxia and hyoptherm ia treatment N=140	Died in neonatal period N=13	Lost to Follow-up N=20	Follow-up age 2 years N=107	Comparison between group of those lost to Follow-up and those who had Follow-up˜
Birth Weight (g), mean (SD)	3451.6 (680.5) min 1540- max 4980	3951.0 (687.7) min 2950- max 4930	3361.1 (708.7) min 1540- max 4625	3375.2 (653.8) min 2200- max 4980	0.5
Gestational age (wk), mean (SD)	39.7 (1.7) min 34- max 42.1	39.7 (1.1) min 37- max 41	39.5 (1.9) min 34- max 42	39.7 (1.6) min 35 -max 42	0.36
Sex, n male/female	67/73	7/6	10/10	50/57	0.16
Mode of delivery n (%) Spontaneous vaginal Forceps Ventouse Planned Caesarean Emergency Caesarean	 56 (40) 12 (8.6) 5 (3.6) 4 (2.2) 63 (45)	 2 (15.4) 1 (7.7) - - 10 (76.9)	 5 (25) 1 (5) 2 (10) - 12 (60)	 49 (45.8) 10 (9.3) 3 (2.8) 4 (3.7) 41 (38.3)	0.10
^Apgar at 10 minutes, mean (SD)	5.04 (2.3) min 0-max 10	3.1 (2.8) min 0-max 9	5.1 (2.5) min 1-max 9	5.3 (2.1) min 0-max 10	0.21
^^Cord PH (arterial), mean (SD)	6.9 (0.2) min 6.5- max 7.3	6.9 (0.1) min 6.6 - max 7.01	6.7 (0.2) min 6.6- mx 7.3	6.9 (0.2) min 6.5 – max 7.3	0.51
^^^Cord Base Excess (arterial), mean (SD)	-15.2 (7.6) min -30 – max +4	-16.1 (7.5) min -28.6 - max -2.4	-13.4 (9.9) min -30 – max -2	-15.4 (7.3) min -30 - max +4	0.12

*Neonatal Seizures, n (%) yes	65 (46.4)	10 (76.9)	6 (30)	49 (45)	0.29

^ Information missing for 1 newborn who died. ^^ Information missing for 11 newborns overall (for 2 newborns who died; for 3 newborns who were lost to FU; for 6 newborns who were seen at age 2 years). ^^^ Information missing for 11 newborns who were seen at age 2 years). * Information missing for 4 newborns overall (for 3 newborns who died; for 1 newborn who was lost to follow-up)

Percentages given are percentages based on available data for each variable in each group.

~ Comparison between the group who was lost to follow-up and the group for which were follow-up information available: Chi-Square and Fisher's Exact test for categorical data, Mann-Whitney U test for continuous data.

Table 2: Results from testing with the Bayley-3 Scales for the 71 children who underwent hypothermia treatment and did not have Cerebral Palsy, categorised by neuromotor status

	Neuromotor status n (%)			
	All tested children n=71* (100%)	Normal n= 61 (85.9%)	Minor neurological signs n= 10 (14.1%)	*p* -value[&]; comparison between group with normal neurology and group with minor neurological signs
***Cognitive composite score, mean (SD), min-max**	106.1 (16.4) min 65-max 145	107.1 (16.8) min 65-max 145	99.5 (11.7) min 80-max120	0.14
**** Language composite score, mean (SD), min-max**	102.9 (15.7) min 59-max 141	104.4 (15.2) min 68-max 141	91.3 (16.3) min 59-max112	0.05
****Language receptive scaled score, mean (SD), min-max**	10.8 (2.6) min 4-max17	10.9 (2.6) min 4-max 17	8.9 (2.1) min 5-max 12	0.04
****Language expressive scaled score, mean (SD), min-max**	10.2 (3.1) min 1-max17	10.4 (3.1) min 2-max17	8.1 (3.3) min 1-max12	0.06
***** Motor composite score, mean (SD), min-max**	105.0 (12.5) min 82-max 136	106.0 (12.3) min 88-max 136	97.5 (12.2) min 82-max 115	0.07
***** Fine motor scaled score, mean (SD)**	10.9 (2.9) min 6-max19	10.9 (2.3) min 6-max19	11.2 (3.5) min 7-max 18	0.84
***** Gross motor scaled score, mean (SD)**	10.7 (2.9) min 6-max19	11.1 (2.8) min 7-max19	8.0 (2.2) min 6- max 12	0.003

* Data available for 71 children; ** data available for 65 children; *** data available for 69 children

[&]Group comparison between normal neurology and minor neurological signs performed with: Mann Whitney U- Test

Table 3: Number of children scoring as normal, borderline or delayed on ASQ, shown by neuromotor status

	Neuromotor status, n	
	Normal n=8	Minor neurological signs n=2
ASQ Communication Skills		
Normal	8/8	2/2
Borderline	0	0
Delayed	0	0
ASQ Gross Motor Skills		
Normal	7/8	1/2
Borderline	1/8	0
Delayed	0	1/2
ASQ Fine Motor Skills		
Normal	7/8	2/2
Borderline	0	0
Delayed	1/8	0
ASQ Problem Solving		
Normal	7/8	2/2
Borderline	1/8	0
Delayed	0	0
ASQ Personal-Social Skills		
Normal	7/8	2/2
Borderline	1/8	0
Delayed	0	0

Table 4: Results from the CBCL 1.5-5 parental questionnaire for children who had TH and did not develop Cerebral Palsy, categorised by neuromotor status

	Neuromotor status n (%)			
	All children with CBCL data n=74 (100%)	Normal n=64 (86.4%)	Minor neurological signs n=10 (13.6%)	*p* -value[&]; comparison between group with normal neurology and group with minor neurological signs
^CBCL Total Problem Score	33.4 (28.6) min 0-max 117	29.1 (26.4) min 0-max 117	56.7 (37.3) min 4-max 108	0.04
^CBCL Internalising Score, mean (SD)	7.3 (7.5) min 0-max-34	5.9 (6.1) min 0-max 31	14.4 (11.9) min 0-max 34	0.03
^CBCL Internalising - Emotionally reactive	2.1 (2.7) min 0-max15	1.7 (2.1) min 0-max 8	4.8 (4.9) min 0-max 15	0.06
^CBCL Internalising - Anxious/depressed	2.1 (2.7) min 0-max 13	1.8 (2.3) min 0-max 13	4.4 (4.1) min 0-max 13	0.04
^CBCL Internalising - Somatic complaints	1.8 (2.3) min 0-max 10	1.5 (1.9) min 0-max 8	3.5 (3.4) min 0-max 10	0.07
^CBCL Internalising - Withdrawn	1.2 (2.1) min 0-max 12	0.97 (1.5) min 0- max 7	1.7 (2.7) min 0-max 8	0.7
^CBCL Externalising Score, mean (SD)	13.1 (10.9) min 0-max 41	12.0 (10.4) min 0-max 41	20.3 (13.3) min 0-max 38	0.09
^CBCL Externalising - Attention	2.8 (2.7) min 0-max 10	2.5 (2.7) min 0 – max 9	3.9 (3.2) min 0-max 10	0.15
^CBCL Externalising - Aggression	10.1 (8.5) min 0-max 33	9.4 (8.2) min 0 – max 33	15.8 (10.1) min 0-max 28	0.06
^CBCL Sleep problems	3.01 (3.7) min 0-max 14	2.6 (3.4) min 0-max 14	5.9 (5.0) min 0-max 14	0.04
^CBCL Other problems	10.1 (8.9) min 0-max 36	8.6 (8.5) min 0-max 36	17.0 (9.9) min 3-max 31	0.01

CBCL, Child Behavior Checklist, raw scores used; data available for 74 children

[&]Group comparison between normal neurology and minor neurological signs performed with Mann Whitney U- Test